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The Genetics of Common Variation affecting Platelet Development, Function and Pharmaceutical Targeting

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Summary

Common variant effects on human platelet function and response to anti-platelet treatment have traditionally been studied using candidate gene approaches involving a limited number of variants and genes. These studies have often been undertaken in clinically defined cohorts. More recently, studies have applied genome-wide scans in larger population samples than prior candidate studies, in some cases scanning relatively healthy individuals. These studies demonstrate synergy with some prior candidate gene findings (e.g., *GP6*, *ADRA2A*) but also uncover novel loci involved in platelet function. Here, I summarise findings on common genetic variation influencing platelet development, function and therapeutics. Taken together, candidate gene and genome-wide studies begin to account for common variation in platelet function and provide information that may ultimately be useful in pharmacogenetic applications in the clinic. More than 50 loci have been identified with consistent associations with platelet phenotypes in ≥2 populations. Several variants are under further study in clinical trials relating to anti-platelet therapies. In order to have useful clinical applications, variants must have large effects on a modifiable outcome. Regardless of clinical applications, studies of common genetic influences, even of small effect, offer additional insights into platelet biology including the importance of intracellular signalling and novel receptors. Understanding of common platelet-related genetics remains behind parallel fields (e.g., lipids, blood pressure) due to challenges in phenotype ascertainment. Further work is necessary to discover and characterise loci for platelet function, and to assess whether these loci contribute to disease aetiologies or response to therapeutics.

Keywords

platelet; aggregation; polymorphism; genome; GWAS; SNP

Importance, measurement and heritability of platelet traits

Platelets play critical roles in thrombosis and haemostasis and are the targets of multiple pharmaceutical targeting strategies and endogenous activation pathways. Severe inherited disorders impacting platelet function have been described underlining the clinical relevance of platelet function (1;2). Evidence further indicates that successful modulation of platelet function provides significant survival benefit, and that variation in platelet volume or function may be an independent risk factor and predictor for disease or outcomes (3–12). Although studies of anti-platelet drug resistance have significant limitations in terms of phenotypic definition, there is general agreement that a significant portion of individuals exhibit anti-platelet resistance (13;14). This, along with the underlying importance of

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Addendum
A. Johnson drafted the manuscript and is solely responsible for its content.

platelet biology and the severity of rare platelet disorders, has provided significant motivation for the study of platelet-related genetics.

This article reviews knowledge on common genetic influences on platelet development, function and pharmaceutical targeting, with particular emphasis on recent developments employing genomic technologies. In particular, a catalogue of >450 platelet-related genome-wide association study (GWAS) results is provided in Supplemental Table 1. Common platelet traits in genetic studies include assays of cell parameters usually performed in whole blood [e.g., platelet count (PLT) and mean platelet volume (MPV)], measurements of platelet function by aggregation response in whole blood or platelet rich plasma (PRP) to a variety of agonists (e.g., collagen), mimetics or stimulants (e.g., shear stress), and less direct measurements of platelet function (e.g., P-selectin levels, fibrinogen levels). Each measurement has potential limitations and focuses most on specific portions of the platelet life cycle or activation cascades.

One gold standard for platelet function testing is light aggregometry in PRP (15), but this measure has a drawback for genetic studies in being challenging to scale to the large sample numbers required to achieve significant statistical power in outbred populations. Cell counts, mainly PLT and MPV in whole blood, are more scalable, and thus have been more widely applied in large genetic studies. While PLT and MPV provide less specific information about platelet function, with modern methods they have reasonable coefficients of variation (16), and the largest genetic study to date on platelet function provided evidence for partial overlap with PLT/MPV loci indicating they may be good proxy measurements for discovering some loci that contribute to platelet function variability (17).

A high degree of heritability for platelet quantitative traits has been widely established, first with twin studies of PRP epinephrine-induced aggregation, PLT and MPV (18;19). Studies on the heritability (h^2) of platelet measures are summarised in Table 1 with the general pattern for h^2 being: MPV > PLT > platelet function tests. A large study of PRP aggregation in families in the Framingham Heart Study (FHS) established the heritability of platelet function to a range of agonists within a general population (20). The Johns Hopkins GeneStar (GS) study later provided further evidence of heritability in both European ancestry and African ancestry families, and to both whole blood (WB) and PRP responses with a range of measures, and in pre- (21) and post- (22) aspirin conditions. Animal model studies, particularly in families of baboons (23) and mouse crosses (24;25), confirm the importance of inherited alleles to platelet measures in other species.

Early studies into the genetics of platelet traits

Early studies into the genetics of specific platelet-related genes largely stemmed from a focus on cells derived from alloimmune thrombocytopenias and efforts to identify the alloantibodies (26). Isolation and sequencing of cDNAs due to the anuclear nature of platelets led to the discovery of coding region polymorphisms. Early variant discovery was restricted by this approach, the nascent state of information on the human genome sequence and infeasibility of sequencing large populations for the coding regions and surrounding sequence. Most early studies focused on cell surface receptors and their ligands, a ***candidate gene*** approach, as these were among the most highly expressed RNAs and proteins with recognised roles in platelet function. A series of coding and proposed regulatory polymorphisms were further studied for their functional effects in association with diseases including venous thrombosis (VT), stroke and myocardial infarction (MI).

A thorough review of platelet candidate gene studies is beyond the scope of this review. Readers are directed to other recent reviews that cover this topic in more depth (26–28). Overall, studies from the candidate gene era did not produce many results that were

consistently replicated in independent populations for roles in either platelet function and/or disease. There are a variety of potential explanations for this observation including the heterogeneity in *ex vivo* assays used in platelet function studies. However, the modest successes of candidate gene studies in platelets are not inconsistent with the experiences of other genetics fields of this era (e.g., blood pressure genetics), and were likely due to limited statistical power in small population samples and the relatively incomplete knowledge of common alleles in human populations available in that time period.

There are a few notable exceptions of platelet candidate genes and polymorphisms that show relatively consistent positive results in the literature including the *GP6* and *ADRA2A* receptors. Another important exception is *CYP2C19*, a cytochrome P-450 enzyme responsible for metabolism of the pro-drug form of clopidogrel, polymorphisms of which have been consistently associated with clopidogrel response. Some candidate genes have re-emerged in genome-wide studies (see below) providing a validated role for these genes in genetic variability (17;29). A similar pattern has been observed for blood pressure loci (30) and other traits (31;32), showing that the information gained from candidate gene studies remains valuable in the genome-wide era.

Genome-wide human linkage and animal QTL studies

First attempts at genome-wide genetic studies for platelet traits employed microsatellite studies to conduct linkage analysis in families, or quantitative trait locus (QTL) mapping in animals. Most linkage analyses use a moderate number of markers and search for cosegregation of markers in individuals' within families with a disease or other trait, generating LOD scores or similar statistics. Results from linkage and QTL studies are summarised in Table 2. While these studies produced several genome-wide significant LOD scores, and even more suggestive signals, in most cases they did not lead to fine mapping, identification or replication of novel platelet function genes. An exception is a linkage study of PLT in Asian Indian pedigrees which fine-mapped putative functional mutations in *THPO* and *GP9* (33). Remarkably, a recent GWAS for PLT in Japanese individuals had a strong peak signal at the same *THPO* variant (rs6141) identified by fine mapping of the linkage scan (34).

There are a few notable consistencies across the human and animal studies. Human chromosome region 19q13.41 was encompassed in linkage peaks for PLT in twins (35), for collagen lag time response in African-Americans (36), and for an orthologous region in the baboon genome in a bivariate analysis of PLT and haemoglobin levels (23). This region contains *GP6*, the direct receptor for platelet activation by collagen, and a previous candidate gene with characterised functional coding alleles. This locus was further validated in a recent GWAS for PRP collagen lag time (17).

Regions orthologous to human chromosome 9q33-q34.3 were identified in mouse PLT studies (25) and in bivariate analysis of MPV and mean corpuscular volume in baboons (23). Notably, this large region of the human genome contains the human blood group antigen locus *ABO*. While this locus has not emerged in large human linkage or genome-wide association studies (GWAS) of PLT, MPV or platelet function to date, some candidate studies have indicated an effect of ABO blood group on particular platelet function measures (37–39). Furthermore, a GWAS study of soluble P-selectin and ICAM-1 levels found the *ABO* locus strongly linked with these measures (40). The effects of ABO-type matching on platelet transfusions may be of potential important to clinical outcomes. A meta-analysis indicates that ABO-matched transfusions consistently result in higher outcome platelet counts (41), though further studies are needed to determine conclusively whether this is of definitive clinical benefit.

Functional genomics studies of platelet traits

Genome-wide genetic studies, transcriptomic (RNA) and proteomic studies have the potential to dramatically advance scientific understanding because they are relatively unbiased and current technologies probe a significant fraction of common human variation, expressed transcripts and proteins. Genome-wide genetic studies have flourished due to extensive maps of common genetic variation (e.g., the HapMap and the 1000 Genomes Project), as well as the stability of DNA and relative availability of samples in large biobanks and cross-sectional population studies. These studies, referred to as GWAS, employ hundreds of thousands or millions of SNP genotypes (or estimates) per individual to conduct large-scale genetic association analysis usually in population-based samples. Transcriptomic and proteomic studies are challenged more than DNA studies by sample availability, technical challenges in assay design and coverage, and the potential impact of confounder variables like tissue specificity and sample quality. In particular, due to the transient platelet life span, challenges in isolation of pure cell populations, and lower transcript and protein copy numbers such studies in platelets face major challenges. Nonetheless, several studies have shown value in non-genetic “omics” approaches to uncovering novel genetic candidates in platelet function.

Platelet endothelial aggregation receptor-1 (*PEAR1*) was a relatively unknown human gene and receptor until it was characterised in a proteomic screen for proteins that undergo tyrosine phosphorylation upon platelet-platelet cell contact (42). Subsequent candidate gene studies consistently found *PEAR1* to be associated with platelet aggregation response to multiple, diverse agonists (43;44), and in both pre- and post-aspirin treated conditions (43). In the largest study of *PEAR1* to date, the combined meta-analysis of PRP aggregation GWAS results from GS and FHS, we found that peak variants in intron 1 of *PEAR1* are strongly associated with both ADP- and epinephrine-induced aggregation (17). Furthermore, we found that intron 1 variants were associated with PEAR1 protein levels in platelet lysates (45). PEAR1, brought to light by proteomics, may be an important novel platelet signalling regulator, a potential drug target and its variants may play a role in platelet-related response variability in the general population, all of which require further detailed study.

Efforts at transcriptome profiling in megakaryocytes and platelets have been undertaken in relatively small sample sizes and often in clinically affected samples, thus our knowledge of transcriptome profiles in the general population is still limited ([n=60 (46); [n=126] (47); [n=29] (48); [n=4 megakaryocyte samples] (49); [n=37] (50)). Despite limited sample sizes, these studies can provide important clues supporting platelet-related genetic signals, or suggest additional candidates for further study. Based on transcript profiling of 37 samples over normal ranges of platelet response, Goodall et al. prioritised several genes for further study, finding that *COMMD7* and *LRRFIP1* show evidence of genetic association with MI (50). In follow-up functional studies employing gene silencing and proteomics approaches *LRRFIP1* demonstrated evidence for effects on thrombus formation and interaction with the platelet cytoskeleton (50).

Notably, of 63 transcripts identified as correlated with platelet responses to ADP and/or a collagen mimetic (50), several are transcribed from loci, or have similar function to loci recently implicated in GWAS for platelet aggregation in PRP (*RGS18*; *ST3GAL3* vs. *ST3GAL4*; *ATP6V0D2* vs. *ATP6V1F* from (17)) and in GWAS for MPV (*DNM3* (51)). Targeted functional reports (e.g., RTPCR) also provide valuable information. In published MPV and PRP GWAS studies the integration of megakaryocyte transcriptome data (49) as well as targeted functional studies on particular genes shows some loci contain transcripts or proteins with known significant expression and/or function in platelets and/or megakaryocytes (17;51); see evidence codes “(R)NA”, “(P)rotein/roteomics”, “(S)ignaling”

in Tables 3 and 4. These examples demonstrate that functional genomics screening experiments can provide valuable information to guide new genetic studies or aid in the interpretation of existing study results.

Human GWAS of platelet traits

A small number of human GWAS studies have been conducted for platelet traits (17;34;36;51–57). The major findings from these studies with consistency in 2 or more population samples are summarised in Tables 3 and 4, along with supporting functional information and citations (e.g., transcriptome, proteome, platelet signalling pathways, animal model results, human disease associations). Table 3 contains loci with evidence for 2 or more platelet phenotypes. Table 4 contains loci mainly associated with a single platelet phenotype. Specific SNP results are not given in Tables 3 and 4 as these can vary across studies due to differences in SNP coverage, population and linkage disequilibrium. Readers interested in further SNP-specific details underlying results in Tables 3 and 4 are referred to Supplemental Table 1 (where >450 significant SNP associations and related SNP identifiers (rsIDs) and mappings are given with reference to the cited GWAS studies).

The first platelet-related GWAS was a moderate density 100,000 SNP scan in FHS which displayed modest findings for PRP aggregation traits with no replication attempted (n≤724, (57)). This study is largely super-ceded by a higher density 550,000 SNP scan in FHS in a larger sample (n≤2,753) with imputation to 2.33 million variants which was further combined in a meta-analysis for similar agonists with imputation-based results from the GS study (n≤2,075) (17). This study represents the only large GWAS study of platelet function, and identified regions strongly associated with PRP ADP response (*PEAR1, MRVI1, SHH*), PRP epinephrine response (*ADRA2A, PEAR1, PIK3CG, JMJD1C*) and collagen lag time (*GP6*). Discovery was conducted in 2 European ancestry samples with replication in 1 African ancestry sample. Thus, this study represents the first platelet-related GWAS study to compare results across ancestry groups. Notably, there is general consistency in signals and direction of effects among many of the loci in both European and African ancestry samples suggesting that most of the platelet function loci may be shared across ancestries (17).

We identified additional loci with consistent associations in 2 or more populations with replication evidence in African ancestry samples but which did not reach genome-significance thresholds. Several loci were associated with platelet responses to 2 or more agonists (Table 3) with the alleles having the same direction of effect in each case, including loci near or containing *ADAMTS2, ATP6VOD2, CTCFL, CUBN, FBXL7, FLJ39743, GMDS, HMG1L1, KIAA0802, KLHL31, MIPOL1, MRVI1, NUP210, PEAR1, PCK1, PIP5K1B, PSKH2, RGS18, SETBP1, STMN4, SVIL, THSD4*, and *WBSCR17*. Overall these results suggest many loci contribute to platelet function, with most contributing to a small proportion of the total population variance.

Many of the identified loci contain genes that were not previously studied in relation to platelet biology (see PubMed search column results for each gene in Tables 3 and 4), indicating the value of GWAS in uncovering novel targets for investigation. Other loci from this study are notable for past links to platelet function including the prion protein *PRNP*, small G-protein related platelet genes including *RAP1B, RAPGEF2, RGS18*, and *PIK3CG*, or for their role in animal models for thrombosis, bleeding or healing (*MRVI1*(58), *PIK3CG* (59;60), *ST3GAL4* (61–63), *RAP1B* (61–64)), or megakaryocyte development or function (*JMJD1C* (65)). The GS cohort published results separately from GWAS of pre- and post-aspirin platelet responses in whole blood and PRP in European ancestry and African ancestry samples (36). These results suggest several potential loci of interest (e.g., the endopeptidase *MME*) but await attempts at replication.

Larger GWAS sample sizes have been achievable for PLT and MPV due to the easier nature of collecting these measures. The first published study conducted GWAS on 335,152 SNPs in 1,606 individuals with MPV measurements, and follow-up replication in up to 8,443 individuals, finding and replicating 3 loci (*ARHGEF3, TAOK1, WDR66*) that account for ~4–5% of the variance in MPV (55). By further investigation of *WDR66* within previously collected microarray results for leukocyte samples, WDR66 transcript levels were inversely correlated with MPV suggesting a functional link. Soranzo et al. followed soon with a MPV GWAS in samples that were used in the replication phase of Meisinger et al., discovering and replicating an additional MPV locus containing *PIK3CG*, and providing evidence that this locus further influences platelet responses to a collagen mimetic as assessed by annexin V binding (56). Consultation with microarray data suggested that *PIK3CG* and another nearby transcript are expressed in megakaryocytes and platelets.

In late 2009 a meta-analysis of MPV and PLT GWAS with up to 4,627 discovery samples and imputation to 2.1 million SNPs and replication in up to 9,316 samples was published (51). This was the first study to conduct GWAS of PLT or to report a platelet-related GWAS that employed SNP imputation to increase genomic coverage. The authors replicated 12 loci for MPV, which included the 4 prior loci, and additionally found and replicated 4 novel loci for PLT (summarised in Tables 3 and 4). Nine of the 12 MPV loci were also associated with PLT; in each case the MPV-raising alleles were associated with an inverse PLT-lowering effect consistent with the inverse correlation of PLT and MPV.

An independent GWAS on up to 6,015 individuals for blood cell traits with imputation to 2.1 million variants found the *HBS1L* locus was strongly associated with PLT (52). Platelet count was the only platelet trait analysed and no PLT SNPs were carried forward to the replication stage. The PLT association with *HBS1L* is consistent with a prior candidate gene study in twins (66), and the strong pleiotropy of this locus including effects on PLT was also observed in prior GWAS (51;53). Given strong association of *HBS1L* with several blood counts it may not be highly specific to platelet function. Ferreira et al. identified several additional suggestive PLT loci in univariate and bivariate analyses including *ARHGEF3*, *PHACTR1*, and *CUBN* (52). The *CUBN* locus is notable since it was subsequently associated with both ADP and epinephrine PRP responses (17) and recently with albuminuria (67). The *PHACTR1* locus is notable since it is one of the published MI GWAS regions (68).

Kamatani and colleagues conducted GWAS of blood measures including PLT in 14,806 Japanese individuals finding support for prior loci (*HBS1L, SH2B3, BAK1, RCL1*) and additionally noting strong associations at 3q27.1 (*THPO*) and 17p13.2 (*GP1B*α) (34). The *THPO* locus encodes thrombopoietin, a known growth factor for MK cells, with the identified GWAS SNP (rs6141) near the translation stop site in the 3'UTR. This region and specific variant were also identified in a prior linkage scan for PLT in Asian Indian families (33). The peak SNP at *GP1B*α (rs6065) encodes a Thr161Met change previously studied as a candidate functional SNP, which was also modestly associated in a GWAS of epinephrine-induced aggregation in PRP (17). Finally, a recent GWAS measured a range of >20 platelet traits in a cohort of children (n=75) with limited replication attempted (54). Despite a sample size that limited statistical power, they discovered and replicated 3 new loci (*LPAR1* for PRP Epi, *MYO5B* for PRP collagen, *NRG3* for PLT), as well as providing support for several prior loci.

Platelet gene stories new and old

In the dawning "omics" era of platelet studies we remarkably find that some of the strongest candidates of prior work now re-emerge among the strongest signals of "omics" work (e.g.,

ADRA2A, GP6, CYP2C19). A number of prior candidate genes also re-emerge with weaker, yet supportive evidence in a recent PRP platelet function GWAS (e.g., *P2RY12, GP1bα*) (17). With more than 50 loci for platelet traits identified, and more than 50 additional PLT/MPV loci promised soon (69), we have considerably advanced in identifying novel candidates for platelet function, as well as ear-marking others that have some prior evidence but have not been the focus of in depth genetic or functional studies. Some have criticised GWAS as not identifying functional variants, but there is evidence that several of the strongest variants from GWAS may be functional (e.g., *GP6, THPO, GP1bα*). Synergy between RNA, protein, animal model, candidate gene and GWAS studies is emerging to varying degrees to identify the likely causal genes within loci and in some cases to identify the potential functional variants.

Among the newly highlighted loci are suggested functional receptors (PEAR1, ST3GAL4, PRNP), potential regulators of cytoskeleton dynamics and platelet morphology (DNM3, TPM1, ARHGEF3), MK development (JMJD1C), and intracellular platelet signalling (PIK3CG, MRVI1 (also known as IRAG), RGS18, RAPGEF2, RAP1B, PTPN11). These candidates propel us beyond a focus on cell surface receptors and their ligands and invite new functional platelet and megakaryocyte studies even without respect to specific alleles. It remains to be seen if any of these loci provide new therapeutic targets, or contribute to a deeper understanding of common or rare thrombosis or haemostasis conditions.

Few of the loci discovered to date have genetic alleles with strongly validated roles in human disease. One exception is *GP6, which* has been independently associated with venous thrombosis in 2 separate studies (70;71). Notably, the peak *GP6* SNP from the VT GWAS and a PRP collagen lag GWAS are the same nonsynonymous SNP which was previously characterised for functional effects. Another exception, *CYP2C19*, is associated with outcomes in anti-platelet treatment trials (29;72–74), though with conflicting results in another set of trials, which could be due to demographic variation among the clinical populations (75). Beyond *CYP2C19* there is no other compelling locus associated with response to aspirin or other anti-platelet therapies at this time (13;76), though *ABCB1* may play a role (73;74). Finally, an extremely large linkage disequilibrium block on chromosome 12q24 has been linked to multiple phenotypes including PLT, blood pressure and coronary artery disease but the functional aetiology and causal gene(s) remains to be worked out (51).

Although most known platelet loci have not been convincingly linked to a platelet-related human condition, evidence from animal models does link some of them to thrombosis or bleeding effects (e.g., *GP6, ST3GAL4, MRVI1, PIK3CG, ADRA2A, P2RY12, RAP1B*). Human disease studies with larger samples and a deeper examination of results with moderate effect sizes may eventually reveal additional platelet-related genes that make modest contributions to disease risk or treatment.

Limitations of current findings and future directions

Current genomic findings are limited both by the constraints of the phenotypes available, the ability to ascertain large populations, and by our current understanding and assessment of human population variation. While variation maps have improved substantially, we are still beginning to survey alleles at the low allele frequency end of the spectrum. Future directions of research include resequencing of platelet loci for additional rare variants that may have larger effect sizes and potential clinical relevance (e.g., in affected families with plateletopathies), functional experiments aimed at uncovering the mechanisms underlying genetic associations, and further study of platelet-related variants in relevant clinical samples which provide adequate statistical power.

While the validation, functional and clinical study of individual variants remains important, locus level views of overlapping trait scans suggests extensive pleiotropy for some platelet-related regions (e.g., *ABO*, *SH2B3*, *HBS1L*). With a growing number of published GWAS and examples of pleiotropy this is an active area of future development since pleiotropic signals may provide important clues to the functions of genes in the associated loci (31;77). As more data become available data mining, pathway analysis and other bioinformatics approaches may also prove useful to characterising important loci for platelet function. We previously assembled a catalogue of results from 118 published GWAS demonstrating the potential to discover novel suggestive patterns (31).

Using a similar, updated catalogue of results from >700 GWAS (unpublished results) Supplemental Table 1 was created with >450 significant associations related to platelet traits from all available GWAS results. This provides a quick overview of loci consistent across studies (e.g., *HBS1L*) but could also suggest loci to prioritise for further study. For example, *RAPGEF2* is seen to be associated with PRP response to ADP in (17) and clopidogrel response assessed by ADP in (29), though this locus has not yet been the focus of replication studies for clopidogrel response. Another potential example is observed when PRP aggregation GWAS, venous thrombosis GWAS and platelet gene expression results are considered. Three distinct loci that encode subunits of a V-type proton ATPase were highlighted: for PRP aggregation associations (*ATP6V0D2*, 8q21.2-q21.3, (17)), gene expression (*ATP6V1F*, 7q23.1, (50)), and in a GWAS follow-up study on venous thrombosis (*ATP6V1B2*, 8p21.2, (78)). These loci were not among the strongest in any of these studies individually. Notably, they are all subunits of a single transporter that has received little study. Evidence indicates this vacuolar ATPase is involved in bone homeostasis (79) and thus it may influence megakaryopoeisis or other platelet-related functions. Additional examples of such hypotheses are likely to emerge as further “omics” studies are published for platelet and haemostasis related traits.

It is important to note that the loci identified thus far which contain common variation contributing to variability in platelet traits individually account for a small proportion of the population variance. Large, well-designed prospective trials of variants are needed to determine their potential relevance in clinical practice. While *CYP2C19* is of potential clinical significance, a position statement does not support routine genotyping or platelet function testing in practice (80).Thus, at this time none of the specific markers highlighted here should be considered to have clear and direct utility in clinical practice.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Heritability studies for platelet-related traits.

Phenotype description	Cohort description	Heritability observations	Citation
Epinephrine-induced PRP aggregation	32 male twin pairs (17 MZ, 15 DZ)	PRP Epi=0.65 in MZ PRP Epi=0.43 in DZ	Gaxiola 1984 (19)
PLT, MPV (Coulter)	92 male and female twin pairs	PLT=0.86, MPV=0.88	Whitfield 1985 (18)
PLT	105 twin pairs	n.s.	Dal Colletto 1993 (81)
PLT (Coulter)	385 twin pairs (192 female, 193 male)	PLT=0.80	Evans 1999 (35)
PLT (Bayer H3 RTX autoanalyzer)	712 twin pairs (234 MZ, 478 DZ)	PLT=0.57	Garner 2000 (82)
Epinephrine, ADP and collagen induced PRP aggregation	1,041 siblings and 928 spouses	ADP=0.44 Epinephrine=0.44 Collagen=0.62	O'Donnell 2001 (20)
PLT	6,111 Italian ancestry individuals in Sardinia	PLT=0.64	Pilia 2006 (83)
Whole blood aggregation (ADP, Collagen, AA, Tbx), PFA closure epinephrine (PFA-100), PRP aggregation (ADP, Collagen, AA, Epinephrine), PLT, MPV	687 European ancestry, 321 African ancestry individuals in families	PLT=0.60/0.67* MPV=0.55/0.71 WB ADP=0.51/0.39 WB Collagen=0.28/n.s. WB AA=0.58/n.s. PFA closure(epi)=0.24/0.61 PRP Collagen=0.26/0.79 PRP Coll. Lag=0.50/0.47 PRP AA=0.38/n.s. PRP Epi=0.36/0.76 PRP ADP=0.42/0.78	Bray 2007 (21)
Post-aspirin responses with or without adjustment of baseline measures: most as above in Bray et al., additionally TXB2 (ELISA), Tx-M (ELISA), beta-thromboglobulin release (ELISA)	1,144 European ancestry, 736 African ancestry individuals in families	PRP Collagen=0.29/0.39*,† PRP Coll. Lag=0.23/n.s. WB Collagen=0.27/0.28 PRP ADP=0.22/n.s. WB ADP=0.20/n.s. PRP Epi=0.33/n.s. PFA closure(epi)=n.s./n.s. TXB2=0.38/n.s. Tx-M=n.s./0.35 B-thromboglobulin=0.33/n.s.	Faraday 2007 (22)
PLT, MPV, PCT, PDW	1,101 Italian ancestry individuals in S. Tyrol	PLT=0.55, MPV=0.79, PCT=0.53, PDW=0.37	Marroni 2008 (84)
PLT, MPV	1,803 Italian ancestry ind. in Val Borbera	PLT=0.60, MPV=0.73	Traglia 2009 (85)
PLT (Coulter)	12,517 individuals in Sardinia	PLT=0.54	Biino 2011 (86)

*Heritabilites are given for the two populations as European ancestry/African ancestry. Where multiple doses were studied the maximum heritability is given.

†Heritabilities under a model fully adjusted for pre-aspirin treatment and other covariates are given. n.s. indicates the authors did not accept the hypothesis that the trait was significantly heritable.

PCT=plateletocrit, PDW=platelet distribution width

Table 2

Human linkage and animal QTL studies for platelet traits.

Phenotype description	Sample description	Human or orthologous human regions (LOD)	Citation
Animal studies			
PLT (Sysmex XE9000 hematology analyzer)	1,126 F2 progeny from a CBA/CaH X QSi5 intercross	Orthologous regions not given. PLT (LODs 11.2, 6.8, 3.6, 3.5, 3.4)	Cheung 2004 (24)
PLT, MPV (Advia 120 whole blood analyzer)	279 F2 progeny each from crosses of NZW/LacJ X SM/J and C57BLKS/J X SM/J	MPV – 12q12-13 (24.2) MPV – 16q13-p21 (2.7) PLT – 9q33-34 (3.5) PLT – 11p15 (3.3) PLT – 2p25 (3.2)	Peters 2005 (25)
PLT, MPV (Coulter)	582 baboons (410 female, 172 male) in 11 pedigrees	PLT/MCV – 1p34.2-p36.23 (3.1) PLT/Hb – 19p13.12-q13.41 (2.2) PLT/MPV – 10q26.3 (2.0) PLT/RDW – 3p23-p22.3 (1.6) PLT/RDW – 10q26.3 (1.5) MPV/RDW – 20p12.1-q11.22 (2.4) MPV/RDW – 10q23.3 (2.0) MPV/MCV – 9q34.2-q34.3 (1.7) MPV/RBC – 8p21.3-p22 (1.6)	Bertin 2007 (23)
PLT, MPV, PDW (CD1700 whole blood analyzer)	1,033 F2 progeny of White Durox X Erhualian porcine crosses	Orthologous regions not given. PLT (F-val 12.1, 10.1) PDW (F-val 21.7, 11.1, 9.6) MPV (F-val 10.1)	Yang 2009 (87)
PLT, MPV, PDW, PCT (TEK-II mini automatic hemocyte analyser)	368 purebred piglet (Landrace, Large White, Songliao Black)	Orthologous regions not given. PLT (LR 17.89, 14.61) PDW (LR 22.24, 19.12)	Gong 2010 (88)
Human studies			
PLT (Coulter)	745 twin pairs (327 MZ, 418 DZ)	PLT – 19q13.11-q13.32 (2.59) PLT – 10q11.23-q22.1 (2.18) PLT – 5q32-q33.2 (1.97) PLT – 2q24.3-q31.1 (1.42) PLT – 5q23.1 (1.26) PLT – 15q11.2 (1.15)	Evans 2004 (35)
PLT (Bayer automated analyzer)	6 Asian Indian families (125 with PLT +markers)	PLT – 3q23 (3.26) PLT – 3q26.2 (2.52)	Garner 2006 (33)
Epinephrine, ADP and collagen induced PRP aggregation	Up to 724 individuals in families	ADP – 7q21.12 (2.0) Collagen – Xp11.4 (2.0)	Yang 2007 (57)
Pre- and Post-aspirin responses with or without adjustment of baseline measures: most as above in Bray et al. Table 1, additionally Tx-M (ELISA)	1,231 European ancestry, 846 African ancestry individuals in families	WB ADP post AA – 5q11.2 (3.6) WB ADP post AA – 5q12.3 (3.6) WB ADP post AA – 5q13.1 (2.2) WB ADP post EA – 5q35.3 (2.2) WB epi post EA – 3p14.2 (2.2) WB epi post EA – 11q23.3 (2.2) WB coll. pre AA – 1q23.3 (2.2) WB coll. post EA – 4p12 (2.2) WB coll. post EA – 4p14 (2.2) WB coll. post EA – 4q12 (2.2) WB coll. post EA – 6q25.3 (2.2) Coll. lag pre AA – 19q13.41 (2.2)	Mathias 2010 (36)

MCV = mean corpuscular volume, Hb = hemoglobin, RDW = red cell distribution width, PDW = platelet distribution width, PCT = plateletocrit

Table 3

Genome-wide association (GWAS) results for platelet traits with consistency in two or more human population samples and association with 2 or more distinct traits in either the same or different studies.

Gene*	Chr region	Phenotype association	Association P-val	Pop.	# in PM†	# in PM (PLT)†	Further evidence
CYP2C19	10q23.33	Clopidogrel response and outcomes[29]	1.5e-13	EA, AA, AS, H	>500	192	DM, FD, HS
PEAR1	1q23.1	PRP Epi, ADP[17], Aspirin PRP response[43], WB coll.[44]	4.9e-19, 3.8e-16, 3.8e-4, 2.6e-4	EA, AA	2	2	P[42;45]
PIK3CG	7q22.3	PRP Epi[17], MPV[51;56;56]	3.1e-9, 1.6e-33	EA	210	13	AM[59;60],R[56],S[59;60]
JMJD1C	10q21.2	PRP Epi[17], MPV[51], PRP ADP[54]	1.6e-8, 3.3e-21, p<0.05	EA, AA	10	0	MK[65]
MRVI1	11p15.4	PRP ADP, Epi[17]	2.0e-8, 7.6e-7	EA, AA	16	2	AM[58],R[89],S[58]
RGS18	1q31.2	PRP Epi, ADP[17]	6.8e-7, 4.0e-5	EA, AA	19	6	R[90–92],P[93], S[93]
TAOK1	17q11.2	MPV[51;55], PRP ADP[17]	1.4e-22, 6.9e-5	EA	3	0	
P2RY12	3q25.1	PRP ADP, Epi[17]	8.2e-6, 9.5e-4	EA	453	395	AM, FD, HS, R, P, S
GP1Bα	17p13.2	PLT[34]	2.1e-12	AS	25	16	AM, FD, HS, R, P, S
		PRP Epi[17]	0.03	EA			
PIP5K1B	9q21.11	PRP ADP, Epi[17]	2.2e-7, 8.0e-6	EA	5	0	
HMGB1L1	20q13.31	PRP ADP, Epi[17]	1.1e-6, 5.2e-6	EA	0	0	
SETBP1	18q12.3	PRP Epi, ADP[17]	1.3e-6, 5.4e-6	EA	8	0	
FBXL7	5p15.1	PRP Epi, ADP[17]	2.9e-6, 2.3e-5	EA	1	0	
ATP6VOD2	8q21.2-q21.3	PRP Epi, ADP[17]	7.9e-6, 8.7e-6	EA	0	0	
KIAA0802	18p11.22	PRP Epi, ADP[17]	9.6e-6, 7.2e-5	EA	0	0	
FLJ39743	15q26.3	PRP Epi, ADP[17]	1.5e-5, 3.3e-5	EA	0	0	
WBSCR17	7q11.22	PRP ADP, Epi[17]	1.7e-5, 6.3e-5	EA	3	0	
CUBN	10p13	PRP ADP, Epi[17]	1.9e-5, 8.1e-5	EA	17	0	
MIPOL1	14q13.3-q21.1	PRP Epi, ADP[17]	2.2e-5, 4.9e-5	EA	5	0	
NUP210	3p25.1	PRP Epi, ADP[17]	4.2e-5, 8.8e-5	EA	44	0	
SVIL	10p11.23	PRP Epi, ADP[17]	4.4e-5, 8.2e-5	EA, AA	17	0	
THSD4	15q23	PRP ADP, Epi[17]	4.4e-5, 8.2e-5	EA	3	0	FD
STMN4	8p21.2	PRP ADP, Epi[17]	5.6e-5, 9.0e-5	EA	23	0	
KLHL31	6p12.1	PRP Epi, ADP[17]	6.0e-5, 6.3e-5	EA	2	0	

Gene*	Chr region	Phenotype association	Association P-val	Pop.	# in PM†	# in PM (PLT)†	Further evidence
GMDS	6p25.3	PRP ADP, Epi[17]	6.1e-5, 8.1e-5	EA	0	0	
ADAMTS2	5q35.3	PRP Epi, ADP[17]	6.8e-5, 7.1e-5	EA	24	0	FD

*some regions contain >1 gene near the genetic signal. Either the closest gene or strongest candidate gene is given.

†2 PubMed search was performed for each gene name on March 16, 2011. One search was only with gene name. The second search was with gene name AND (platelet OR megakaryocyte OR thrombosis OR hemostasis). The GWAS studies considered here are not counted among the results.

Population abbreviations are: EA=European ancestry, AA=African ancestry, AS=Asian ancestry, H=Hispanic. Further evidence abbreviations: AM=animal model, DM=drug metabolism function, FD=functional domain, HS=human study of platelet function/disease, MK=role in megakaryocyte development or function, P=protein or proteomics evidence for a role in platelets/MK, R=RNA gene expression evidence for role in platelets/MK, S=signaling role known in platelets/MK

Table 4

Genome-wide association (GWAS) results for platelet traits with consistency in two or more human population samples with strong association with only one trait.

Gene*	Chr region	Phenotype association	Association P-val	Pop.	# in PM†	# in PM (PLT)†	Further evidence
ADRA2A	10q25.2	PRP Epi[17]	3.2e-12	EA, AA	>500	>500	FD, HS[94], R[95], P[96]
NEURL	10q24.33	PRP Epi[17]	2.5e-7	EA, AA	10	1	
PRNP	20p13	PRP Epi[17]	1.7e-6	EA, AA	>500	1	HS[97], P[98]
TRIM27	6p22.1	PRP Epi[17]	2.4e-6	EA, AA	54	0	
SGCZ	8p22	PRP Epi[17]	4.5e-6	EA, AA	2	0	
ST3GAL4	11q24.2	PRP Epi[17]	3.0e-6	EA, AA	5	1	AM[61;63], FD[62], HS[61]
LPAR1	9q31.3	PRP Epi[54]	5.8e-3	EA	11	0	FD, HS[99]
SHH	7q36.3	PRP ADP[17]	4.5e-8	EA, AA	>500	34	MK[100]
MST151	10p13	PRP ADP[17]	6.7e-7	EA, AA	0	0	
KCNQ1	11p15.5-p15.4	PRP ADP[17]	5.9e-6	EA, AA	200	0	
RAPGEF2	4q32.1	PRP ADP[17]	9.1e-7	EA, AA	19	6	S[101;102]
JAK2	9p24.1	WB ADP[44], PRP ADP[54]	6.0e-4, p<0.05	EA	>500	499	AM, FD, HS, R, P, S
COMMD7	20q11.21	WB ADP[50], PRP ADP[54]	1.2e-3, p<0.05	EA	0	0	AM, R[50]
GP6	19q13.42	PRP collagen lag time[17]	8.4e-14	EA, AA	237	17	FD, R[103], P[104]
PTPRD	9p24.1	PRP collagen lag time[17]	1.2e-7	EA, AA	200	3	
HSD17B6	12q13.3	PRP collagen lag time[17]	1.1e-6	EA, AA	6	0	
FCER1G	1q23.3	WB coll.[44], PRP coll. lag[17]	9.6e-6, 1.6e-5	EA	12	0	
UGT1A10	2q37.1	PRP collagen lag time[17]	1.2e-5	EA, AA	126	0	
RAP1B	12q15	PRP collagen lag time[17]	1.5e-5	EA, AA	150	52	AM[64], R[105;106], S[107], P[108]
MYO5B	18q21.1	PRP collagen[54]	3.0e-5	EA	34	0	
WDR66	12q24.31	MPV[51;55]	2.7e-44	EA	0	0	
ARHGEF3	3p21-p13	MPV[51;55]	5.5e-31	EA	6	0	R[109]
TMCC2	1q32.1	MPV[51]	1.4e-20	EA	1	0	
BET1L	11p15.5	MPV[51]	1.3e-14	EA	10	0	
DNM3	1q24.3	MPV[51]	2.1e-14	EA	6	1	MK[110], R[50]

Gene*	Chr region	Phenotype association	Association P-val	Pop.	# in PM†	# in PM (PLT)†	Further evidence
EHD3	2p21	MPV[51]	3.2e-11	EA	21	0	R[51]
SIRPA	20p13	MPV[51]	7.7e-11	EA	193	2	
CD226	18q22.3	MPV[51]	1.4e-10	EA	113	0	MK[111]
TPM1	15q22.1	MPV[51]	1.9e-8	EA	158	1	MK[112], R[51;51;112]
AK3	9p24.1-p24.3	PLT[51]	8.5e-17	EA	51	0	
ATXN2	12q24	PLT[51]	2.2e-13	EA	23	0	R[51]
PTPN11	12q24	PLT[51]	7.7e-12	EA	200	108	HS[113], R[51], S[113;114]
THPO	3q27.1	PLT[34]	5.4e-11	AS	94	12	MK[115], FD, HS[33;116]
BAK1	6p21.3	PLT[51]	3.7e-10	EA	>500	10	
NRG3	10q23.1	PLT[54]	3.6e-5	EA	38	0	

*some regions contain >1 gene near the genetic signal. Either the closest gene or strongest candidate gene is given.

†2 PubMed search was performed for each gene name on March 16, 2011. One search was only with gene name. The second search was with gene name AND (platelet OR megakaryocyte OR thrombosis OR hemostasis). The GWAS studies considered here are not counted among the results.

Population abbreviations are: EA=European ancestry, AA=African ancestry, AS=Asian ancestry. Further evidence abbreviations: AM=animal model, FD=functional domain, HS=human study of platelet function/disease, MK=role in megakaryocyte development or function, P=protein or proteomics evidence for a role in platelets/MK, R=RNA gene expression evidence for role in platelets/MK, S=signaling role known in platelets/MK